in physical activity, diet, and other factors. We examined myofilaments' contractile characteristics and physical performance, walking speed and climbing rate, in African green vervet monkeys, housed in social groups in large indoor-outdoor enclosures and fed the same diet. Physical performance and skinned vastus lateralis (VL) muscle fiber function were investigated in four young (11 ± 1 yrs) and four old (23 ± 1 yrs) monkeys. Fiber myosin heavy chain (MHC) isoform was determined by gel electrophoresis. The old monkeys walked slower (19%) and climbed less (63%) than young monkeys ($p<0.05$). Myofiber cross sectional area (CSA) was 22% and 12% smaller for IIa and hybrid MHC, respectively, in old compared to young monkeys ($p<0.001$). Specific force (maximal Ca^{2+}-activated force normalized to fiber CSA) was 15% and 11% less for type IIa and hybrid fiber, respectively, in old compared to young monkeys ($p<0.05$). Fiber atrophy does not account for the loss in force with aging; it declined much faster than fiber CSA. Although we observed no difference in shortening velocity, the maximal power output substantially decreased in 21% of type IIa and 22% of hybrid fibers with aging ($p<0.05$). Regression modeling used to identify factors contributing to lower fiber force revealed that age is the strongest predictor (r^2=0.31, $p<0.001$). The diminished contractile properties measured *in vitro* correlates strongly with age-dependent decline in physical performance (waking speed: r = 0.41, p < 0.001); climbing rate: r = 0.29, p < 0.001). Our results support a detrimental effect of aging on the innate force and power generation of myofilament lattice and physical performance.

730-Pos Board B516

Slow Myosin ATP Turnover in the Super-Relaxed State in Tarantula Muscle

Edward Pate[1], Nariman Naber[2], Roger Cooke[2].
[1]WSU, Pullman, WA, USA, [2]UCSF, San Francisco, CA, USA.

We measured the nucleotide turnover activity of myosin in tarantula leg-muscle fibers by observing single turnovers of the fluorescent nucleotide analog, mantATP, as monitored by the decrease in fluorescence when mantATP is replaced by ATP in a chase experiment. We find a multi-exponential process, with approximately two-thirds of the myosin showing a very slow nucleotide turnover time constant, ~30 minutes. This slow turnover state is termed the super-relaxed state (SRX) and is a highly novel adaptation for energy conservation in an animal that spends extremely long periods of time in a quiescent state (days) employing a lie-in-wait hunting strategy. If fibers are incubated in mantADP and chased with ADP, the SRX is not seen, indicating that relaxed myosins are responsible for the SRX. Phosphorylation of the myosin regulatory light chain eliminates the fraction of myosin with the very long lifetime. The presence of the SRX measured here correlates well with the binding of myosin to the core of the thick filament in a structure known as the interacting-head motif (or J-motif) observed previously by electron microscopy. Both the structural array and the long-lived SRX require ATP, both are lost upon myosin phosphorylation, and both appear to be more stable in tarantula than in skeletal or cardiac preparations. EPR spectroscopy of a spin-labeled nucleotide bound to the motor domain of myosin in relaxed tarantula fibers likewise shows orientation that is lost when the myosin is phosphorylated. Together, the data support the hypothesis that the SRX myosin and the myosin seen in the EM of the order helical array in tarantula filaments are the same.

731-Pos Board B517

The Low Angle X-Ray Diffraction Pattern from Skinned Fibers of Rabbit Psoas Muscle: Effect of Changes in [Ca^{++}] and [Orthophosphate]

Elisabetta Brunello[1], Marco Caremani[1], Massimo Reconditi[1], Marco Linari[1], Mario Dolfi[1], Manuel Fernandez Martinez[2], Theyencheri Narayanan[2], Gabriella Piazzesi[1], Malcolm Irving[3], Vincenzo Lombardi[1].
[1]University of Florence, Florence, Italy, [2]ESRF, Grenoble, France, [3]King's College London, London, United Kingdom.

Bundles of 3-5 fibers were activated isometrically at different pCa by a temperature jump from 1°C to 12°C using a mechanical apparatus (Linari *et al*., Biophys. J. 92:2476, 2007) modified to collect the X-ray diffraction pattern. The M3 meridional reflection from the axial repeat of the myosin heads was sampled by X-ray interference between half-sarcomeres. In relaxed fibers at 12°C, the M3 reflection had a major peak at 14.56 nm and a minor peak at 14.37 nm. The ratio of peak intensities (R_{M3}) was 0.43 ± 0.06 and the spacing (S_{M3}) was 14.49 ± 0.01 nm. In relaxed fibers the intensity of the main peak reduced with increasing temperature, so that at 36°C (the physiological temperature) the 14.37 nm peak was dominant, with small satellite peaks on either side, as in resting intact fibers from frog muscle. During activation at 12°C at saturating [Ca^{++}], pCa 4.5, the intensity of the M3 reflection (I_{M3}) increased to 1.9 ± 0.4 times the relaxed value with major and minor peaks at 14.68 nm and 14.46 nm; R_{M3} was 0.62 ± 0.03 and S_{M3} was 14.59 ± 0.01 nm. Activation at pCa 5.5 or at pCa 4.5 with addition of 10 mM orthophosphate (Pi) had similar effects: force was reduced to 0.34 ± 0.10 the control value and I_{M3} to 0.56 ± 0.03; R_{M3} was 0.46 ± 0.07 and S_{M3} was 14.55 ± 0.02 nm. These results give structural support to the conclusion from mechanical experiments (Linari *et al*., 2007) that both decreasing [Ca^{++}] and increasing [Pi] reduce isometric force by a decrease in the number of force generating myosin heads with no change in force per head.

Supported by FIRB-Futuro in Ricerca and Ente Cassa di Risparmio di Firenze (Italy), MRC (UK).

732-Pos Board B518

Structural Changes in Myosin Heads and Filaments during Unloaded Shortening and Force Redevelopment

Massimo Reconditi[1], Elisabetta Brunello[1], Marco Linari[1], Luca Fusi[1], Theyencheri Narayanan[2], Gabriella Piazzesi[1], Malcolm Irving[3], Vincenzo Lombardi[1].
[1]University of Florence, Florence, Italy, [2]ESRF, Grenoble, France, [3]King's College London, London, United Kingdom.

X-ray diffraction patterns were recorded with 5-ms time resolution at the ID02 beamline, ESRF, from single intact muscle fibers of the frog during steady shortening at the maximum velocity V_0, imposed at the plateau of an isometric tetanus (T_0), and during isometric force redevelopment following such shortening. During the first 20nm/half-sarcomere (hs) of shortening force decreased to near zero and changes in the X-ray pattern were consistent with a working stroke in actin-attached myosin heads followed by net detachment from actin (Piazzesi *et al*., *Cell* **131**:784, 2007). As V_0 shortening continued the M3 meridional reflection (associated with the conformation of the myosin heads) and its second order M6 (associated with the thick filament structure) became more like those recorded at rest. At 110 nm/hs shortening the M3 spacing and its fine structure were the same as at rest, while the M3 intensity, M6 spacing, and intensity of the first myosin layer line from the helical packing of the myosin heads, had recovered about half-way to their resting values, without sign of saturation. Isometric force redevelopment following 110 nm/hs shortening at V_0 and the associated structural changes were faster than those at the start of electrical stimulation (Reconditi et al., *PNAS*, **108**:7236, 2011). In both cases the initial force generation involves a small fraction of the myosin heads, whilst the majority are in the resting-like helically ordered conformation on the surface of the thick filament. The relationship between force and structural change is the same in the two cases for forces above 40% T_0. The rates of the structural changes at the start of stimulation are limited by the rate of thin filament activation.

Supported by MiUR and FIRB - Futuro in ricerca (Italy), MRC (UK), ESRF.

733-Pos Board B519

Sarcomere-Length Dependence of the Low Angle X-Ray Pattern from Skeletal Muscle Fibers at Rest and during Isometric Contraction

Gabriella Piazzesi[1], Massimo Reconditi[1], Elisabetta Brunello[1], Luca Fusi[2], Marco Linari[1], Manuel Fernandez[3], Theyencheri Narayanan[3], Vincenzo Lombardi[1], Malcolm Irving[2].
[1]University of Florence, Florence, Italy, [2]King's College London, London, United Kingdom, [3]European Synchrotron Radiation Facility, Grenoble, France.

X-ray patterns were recorded from bundles of 2-3 fibers of *R. Esculenta* at 4°C at rest and at the plateau of an isometric tetanus (T0) at sarcomere length (SL) 2.0 to 3.6 μm at the ID02 beamline at the European Synchrotron Radiation Facility, Grenoble. The patterns were normalized by the intensity of the 1,0 equatorial reflection at rest at each experimental SL to compensate for variation of diffracting mass with SL and between fibers. The axial diffraction pattern from resting fibers was independent of SL in the range 2.0-2.6 μm; in the range 2.6-3.0 μm the intensity and interference fine structure of the meridional M3 reflection from the axial repeat of the myosin heads along the filaments was constant, but its spacing (SM3) increased. The intensity of the first myosin layer line decreased in this SL range, indicating decreased helical ordering of the myosin heads. The intensity of the 44nm merdional reflection associated with myosin binding protein C was constant up to SL 2.7 μm, but much reduced for SL>2.7 μm. At T0, the M3 reflection intensity was smaller at longer SL, in proportion to the overlap between thick and thin filaments. The interference fine structure of the M3 was independent of SL up to 2.8 μm; at longer SL it varied between preparations and SM3 reduced with increasing SL. The SL-dependence of the M3 reflection at T0 indicates that the detached myosin heads in the non-overlap region of the thick filament are axially disordered compared with actin-attached heads in the overlap region, although the axial center of mass of the detached

and attached heads is similar. Supported by Ente Cassa di Risparmio di Firenze, FIRB-Futuro in Ricerca, MRC and ESRF.

734-Pos Board B520

Role of Pro-Ala-Rich Extension of Troponin in Insect Flight Muscle as Examined by X-Ray Diffraction

Hiroyuki Iwamoto, Naoto Yagi.
Spring-8, JASRI, Sayo-gun, Hyogo, Japan.

The mechanism of stretch activation is essential for the asynchronous action of insect flight muscle (IFM). Our previous study has shown that the 1,1 row-line spot on the 1st layer line reflection from bumblebee IFM is the first one to respond to the stretch signal in a calcium-dependent manner, and the model calculation has suggested that it may come from the structural change of troponin. This makes IFM troponin a candidate for the stretch sensor. Then the question is what structure transmits stretch information to troponin. IFM troponin-I (troponin-H) is known to have a long Pro-Ala-rich extension, and this may extend to the thick filament and pick up the stretch information. To test this possibility, the extension was severed by a specific endoproteinase (Igase) in bumblebee IFM, and its mechanical properties and X-ray diffraction patterns were examined. In honeybee troponin-H, the susceptible sequence is located at the base of the extension. Unexpectedly, the ability of stretch activation was not affected. The severing had a dramatic effect on diffraction patterns: In IFMs in general, the equatorial 2,0 reflection is much more intense than the 1,1 reflection, but after Igase treatment, the intensities of these reflections became almost equal. If this change is caused by the reduced mass of the thin filament, its mass should be reduced to 1/4 while the extension accounts for only 10% of the thin filament mass. In the SDS gel pattern, the 80-KDa band for troponin H disappeared from the fiber and several protein bands appeared in the solution after treatment. A possibility is that each extension binds several GST molecules (Clayton et al., 1998) to process reactive oxygen generated by the high mitochondrial activities in IFM cells.

735-Pos Board B521

Modeling the Working Stroke of the Muscle Crossbridges

Hugh Huxley[1], Massimo Reconditi[2].
[1]Brandeis univ., Waltham, MA, USA, [2]Physiology Department, University of Florence, Florence, Italy.

It has been suggested by Knupp *et al*. (*J.Mol.Biol.* **390:**168–181, 2009) that the lever arms of the myosin crossbridges (subfragment 1) make virtually no contribution to the behavior of the M6 meridional reflection, and that recent X-ray interference studies provide no evidence for lever arm tilting during the working stroke. Instead, it is claimed that the observed changes can be predicted by models in which the working stroke involves a length change of subfragment 2, with the lever arm remaining in either the Dominguez or Rayment configuration (the conventional pre- and post-working stroke positions), or by models in which a changing mixture of these configurations is present during the stroke.

In our previous work (Huxley *et al*. *J.Mol.Biol.* **353:**743–761, 2006), we showed that the relative intensities of the M3 and M6 reflections and of the inner and outer interference peaks of M6 require that in isometric contraction the crossbridge lever arms must be angularly dispersed through $\pm 20^\circ$ or more, and that a second component, presumably in the myosin backbone, must contribute to M6 to shift the phase of the interference pattern appropriately. The observed steady increase in the M6 intensity, by 30-40% or more, with releases up to~7 nm, and then a small decrease, is modeled satisfactorily with the tilting lever arm. However, if the modeling involves a fixed lever arm angle (either Dominguez or Rayment, or a mixture of the two configurations) with an S2 of varying length, the predicted M6 reflection undergoes several large swings in intensity over the relevant range of releases, and bears no relation to what is observed. This shows that the lever arm configuration plays a major role in determining the M6 behavior, and that the data support a straightforward tilting lever arm model.

736-Pos Board B522

Length Dependent Activation in Synchronous Flight Muscle from Manduca Sexta

Mohit Kumar, Peter Schemmel, Chen-Ching Yuan, **Thomas C. Irving**.
Illinois Institute of Technology, Chicago, IL, USA.

In most striated muscles, the amount of force generated at a given concentration of activating calcium is greater at long sarcomere length (SL) than at shorter SL, referred to as ''length dependent activation'' (LDA). LDA is prominent in mammalian cardiac muscle and underlies the so-called Frank-Starling Law of the Heart'' allowing cardiac output to be adjusted on a beat-to-beat basis. The molecular basis for LDA is not yet understood. The dorsal longitudinal flight muscle (DLM) of the Hawkmoth *Manduca sexta* is an emerging model system for structural and functional studies of muscle. It is a synchronous muscle, requiring a neural impulse for every muscle twitch, as in mammalian skeletal and cardiac striated muscle, but it is structurally similar to the more widely studied asynchronous insect flight muscles of *Drosophila* and *Lethocerus*. Its force-length curve has been shown to be remarkably similar to mammalian cardiac muscle (Tu & Daniel, *J Exp Biol 207*: 2455. 2004) indicating that Manduca flight muscle might be a useful model system to elucidate various aspects of cardiac function in comparative studies. The present studies were undertaken to characterize LDA in *Manduca* flight muscle. Conditions were found that allowed chemical skinning of the muscles while maintaining good structural order as assessed by by light and X-ray diffraction. Force-pCa curves were collected as a function of SL. Dorsally located DLMs (cooler in vivo) were compared to ventrally located DLM's (warmer in vivo). We found that both dorsal and ventral DLMs show length-dependent activation. Our study also showed that ventrally located DLM's are less cooperative (Hill coefficient n_H ~1-1.2) than the dorsally located DLM's (n_H ~1.8–1.9) which may be related to their different functions in vivo. Supported by NSF IOS 1022058 and NIH RR08630.

737-Pos Board B523

The Origins of Cross-Bridges in Active and Rigor Insect Flight Muscle are not as Predicted from Acto-S1 and X-Ray Crystallography

Claudia L. Vargas[1], Anthony Warrington[1], Kenneth A. Taylor[1], Susan Hester[2], R.J. Perz-Edwards[2], Michael K. Reedy[2].
[1]Florida State University, Tallahassee, FL, USA, [2]Duke University, Durham, NC, USA.

In fast-frozen, actively contracting insect flight muscle (IFM) fibers we noted a surprising azimuthal slew in the myosin lever arms that was wholly unexpected from the currently available crystal structures of isolated myosin S1 and which indicates the crystal structures may not reflect the normal in situ constraints within the sarcomere where myosin cross-bridges are restricted to originate from a well defined zone on the thick filament [Wu et al., 2010, PLoS-ONE, 5(9): e12643]. However, previous studies did not reveal the S2 domains, which most accurately define the myosin head origin. Here, we have used electron tomography (ET) of Lethocerus IFM fibers in rigor in which the filament lattice has been swollen in low ionic strength buffer to view the S2 origins of rigor ''lead bridges''. These rigor lead bridges bind to the same region of the thin filament as myosin heads of active muscle so their origins should reflect the same thick filament positions as active heads. We examined 80 nm thick transverse sections cut with a vibrating knife to minimize section compression and shearing artifacts, and imaged with < 60 e-/Å2 total exposure during each tilt series to minimize radiation-induced section thinning. This analysis shows two different distributions of lead bridge origins depending on the presence of the rear bridges. However, the origins are consistent with target zone accessibility of strong binding myosin heads being limited to two successive 14.5 nm crowns. Subvolume averages of both thick filaments as well as actin filaments are being pursued with the goal of reassembling a region of the filament lattice using high S/N averages. Supported by NIGMS and NIAMSD.

738-Pos Board B524

Interaction Between the Relay Loop and the SH1-SH2 Helix Region in Drosophila Muscle Myosin is Essential for Normal Motor Function, Myofibril Stability and Muscle Contraction

Girish C. Melkani, William A. Kronert, Anju Melkani, Sanford I. Bernstein.
Department of Biology, Molecular Biology Institute, and SDSU Heart Institute, San Diego State University, San Diego, CA, USA.

Molecular modeling of *Drosophila* muscle myosin reveals that relay domain residue E499 interacts with SH1-SH2 residue R714 in a charge-dependent manner. To explore the significance of this interaction, we generated transgenic lines expressing myosin with a mutation in the relay loop domain (*E499R*) or the SH1-SH2 helix (*R714E*). Both mutations yield ~75% reductions in CaATPase as well as basal or actin-activated MgATPase activity. Actin-sliding velocity of *E499R* is reduced by 65% and *R714E* shows no motility. Indirect flight muscles in late pupae of each mutant display disrupted myofibril assembly. Two-hour- and two-day-old adults have severely abnormal myofibril morphology and poor sarcomere organization, with no flight ability. Therefore, the putative interaction of the relay with SH1-SH2 is indispensable for normal motor function, myofibril stability and locomotion. We next constructed a putative compensatory mutant designed to restore function of the *E499R* or *R714E* mutant by generating a transgenic line that expresses both *E499R and R714E*. Interestingly, calcium, basal, and actin-stimulated ATPase values are restored to 65–70% of wild type and actin-sliding velocity is at 40%.